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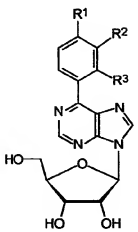
(54) Title: NOVEL 6-PHENYLPURINE 9-β-D-RIBONUCLEOSIDES WITH ANTINEOPLASTIC ACTIVITY, THEIR USE FOR PREPARING PHARMACEUTICAL COMPOSITIONS AND PHARMACEUTICAL PREPARATIONS CONTAINING SUCH COMPOUNDS



(I)

(57) Abstract: Novel 6-phenylpurine 9-β-D-ribonucleosides with antineoplastic activity of general formula (I), wherein R^1 is H, methyl, fluoro, chloro or alkoxy (C1-C2) group, R^2 is H, methyl or fluoro group, and R^3 is H, fluoro or methoxy group. Pharmaceutical compositions containing the compounds of the invention as active components are also described. Further the use of compounds of general formula (I) according to Claim 1, or their combinations, for preparing pharmaceutical compositions for the treatment of cancer and leukemia is described.

WO 00/75158 A2

Description

Novel 6-phenylpurine 9-β-D-ribonucleosides with antineoplastic activity, their use for preparing pharmaceutical compositions and pharmaceutical preparations containing such compounds

Technical Field

The present invention relates to novel compounds and compositions which have antineoplastic activity, processes for making such compounds, use of such compounds as active components of pharmaceutical preparations and pharmaceutical preparations containing such compounds.

Background Art

Malignant tumors, various forms of leukemia and related disturbances of hematopoietic system belong to main causes of human morbidity and mortality. According to WHO data, these neoplasias amounted to >12% of global mortality in 1996 and their proportion in civilized countries is substantially higher. Among the most dangerous forms, there are lung carcinomas, hepatomas, osteosarcomas, cancers of stomach, colon and rectum, ovaries, breast and uterus. In the Czech Republic the mortality due to various forms of cancer amounts to >20% of deaths.

Chemotherapy is an essential part of the cancer therapy. In most cases it complements surgical and palliative therapy; however, in certain cases (e.g. leukemia) it is the main therapeutic method. Chemotherapy is also often very efficient in the treatment of early stages of some cancers: the most sensitive are Hodgkin's lymphoma, seminoma, ovarian cancer, small-cell lung carcinoma, skin cancers and prostate cancer. Also the carcinoma of cervix is reportedly chemotherapy-responsive. Particularly important is the chemotherapy for treatment of acute lymphoblastic leukemia in children and acute myeloblastic leukemia of adults.

Strategy of chemotherapeutic attitudes in the treatment of malignancies makes use of molecular biological principles of cell transformation and other processes taking place in a transformed cell; it is supplemented by additional methods influencing tumor angiogenesis, hormone equilibria in the organism, etc. Chemotherapy of neoplasias is subject of numerous monographs (from the recent

books e.g. O.Foye, Cancer Chemotherapeutic Agents; ACS, Washington, D.C.1995; P.Klener, Protinádorová chemoterapie, Galén, Praha 1996) and specialized scientific and clinical journals.

Among the most important clinically used anticancer drugs are e.g. antimitotic drugs (vincristine, paclitaxel, podophyllotoxin), alkylation agents – nitrogen mustards (chlorambucil, melphalan, manomustin, cyclophosphamide, ifosfamide, mafosfamide), aziridines (tretamine, thio-TEPA, mitomycins), sulphonates (busulfan), platinum complexes (cisplatin, carboplatin) and nitrosoureas (semustine, lomustine, carmustine, streptozotocin), topoisomerase inhibitors (teniposide, etoposide, irinotecan, merbaron), radiomimetics (bleomycins, streptonigrin, neocarcinostatin) and intercalators, e.g..anthracycline antibiotics (daunorubicin, doxorubicin, aclarubicin, zorubicin, idarubicin, etc.), chromomycins, anthracene derivatives (mitoxanthrone, amethanthrone) and others.

Antimetabolites mimicking the structure of natural metabolites are important anticancer chemotherapeutics. They affect the enzymatic reactions wherein the metabolites participate. Consequently, these drugs cause inhibition of important metabolic pathways or production of altered metabolic products lacking the required properties. Thereby, the key-processes important for cell replication during cell mitosis, transcription of DNA to RNA or the following translation (protein formation) are limited or made impossible or, the enzymatic reactions important during the cell life cycle are limited and/or damaged.

Antineoplastic or antileukemic drugs based on such antimetabolic principles are e.g. folic acid analogs (methotrexate, trimetrexate, edatrexate, Tomudex, Thymitaq), ribonucleotide reductase inhibitors (hydroxyurea, guanazole, ceracemide), amino acid analogs (DFMO, PALA) and numerous drugs interfering with nucleic acid metabolism (synthesis and function of their precursors, polymerisation reactions from monomers). Clinically used cancerostatics and antileukemic drugs are often pyrimidine or purine derivatives. Thus, among pyrimidine derivatives, particularly uracil analogs (5-fluorouracil and its prodrugs, e.g. togafur, floxuridin), cytosine derivatives (cytosine arabinoside, cyclocytidine, gemcitabine), 5-azacytosine derivatives (decitabine, fazaribine). The main clinically used purine derivatives are mercaptopurine and its congeners (thioguanine, azathioprin) and adenosine analogs (adenine arabinoside, deoxycoformycin, cladribine and fludarabine).

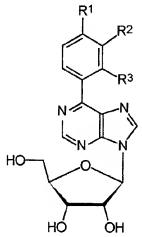
The present successful clinical attitudes for chemotherapeutical treatment of malignant diseases are always making use of a combination based on numerous active components. The main problem of such a therapy is drug toxicity and multiple side reactions which are due to the fact that the most principles used for design of antineoplastic drugs apply both to transformed as well as to normal cells. Thus, only relative differences (e.g. increased mitotic activity in some transformed cells, hypoxic character of tumor tissue) or unique properties of the pathological condition (metastatic activity, increase need for angiogenesis) can be taken in account. The success of drug combination therapy is further limited by multiple drug resistance (MDR), a natural process which diminishes the drug efficacy by an increased drug efflux from the cell. These disadvantages can be circumvented by continuous introduction of novel drugs based on known – but preferably original - principles of biological interference with metabolic pathways of transformed cells, with metastasis, tumor formation, etc.

The prerequisites of a successful application of a antineoplastic drug candidate are its suitable pharmacological parameters as well as its sufficient biological stability which is immediately connected both with a need for more frequent drug administration and with a potential toxicity of drug catabolism (decomposition) products. Such a situation occurs frequently with the drugs based on close structural similarity to the natural substrates of enzymes, including enzymes involved in catabolic reactions. Thus, the use of cytosine arabinoside in treatment of leukemia is limited by its deamination to inactive uracil arabinoside; similar processes occur with gemcitabine, while limiting factor for fazarabine is its chemical lability *per se*. Since some catabolic processes can be efficiently inhibited, it is possible to apply in a combination with the drug the appropriate inhibitors of, e.g., uridine phosphorylase, cytosine deaminase (with 5-fluorocytosine), adenosine deaminase (deoxycoformycin with adenine arabinoside, cladribine or fludarabine). The design of drugs, which cannot principally undergo such catabolic transformations, is indeed preferable.

Such a possibility offers the present invention of novel 6-phenylpurine 9-β-D-ribonucleosides with antineoplastic activity.

Disclosure of Invention

The subject of the present invention are novel 6-phenylpurine 9-β-D-ribonucleosides with antineoplastic activity of the formula (I):



(I)

wherein R^1 is H, methyl, fluoro, chloro or alkoxy (C1-C2) group, R^2 is H, methyl or fluoro group, and R^3 is H, fluoro or methoxy group.

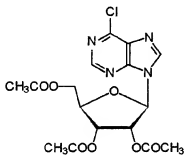
The present invention also comprises the use of compounds of the general formula (I) or their combinations as active components of pharmaceutical preparations for treatment of cancer and leukemia, as well as the use of such pharmaceutical preparations.

Among purine derivatives with modified heterocyclic purine base, there are numerous compounds displaying various biological activities. In addition to the above-mentioned cladribine or fludarabine containing 2-chloroadenine or 2-fluoroadenine base, cytostatic activity is known for 7-deazapurine derivatives cadeguomycin a 7-deazainosine, antimetastatic effect for 7-deazaguanosine. 8-Aza-guanosine and 8-azainosine are cytostatics, while 3-deazaguanosine exerts an antitrypanosomal effect. Loxoribine (7-allyl-8-oxoguanosine) is immunostimulatory, while 2-alkenyladenosines are A2 receptor agonists with significant antihypertensive activity. Though a certain biological activity of 2-arylpurine nucleosides (connected with their effect on purinoceptors) was recorded in the literature, the only existing mention of 6-phenylpurine nucleoside (Bergstrom D.E., Reddy P.A., Tetrahedron Lett. 1982, 23, 4191), unsubstituted 6-phenylpurine 9-β-D-ribonucleoside, lacks any information about its biological activity. Thus, the discovery of cytostatic activity connected with the compounds of general formula (I) modified by diverse

substituents at the 6-aryl group is quite unexpected; it cannot be deduced from any analogy either with the known purine nucleosides exerting cytostatic activity, or with purine nucleosides bearing diverse 6-substituents. The observed cytostatic effect is characteristic for the combination of 6-phenylpurine bearing certain substituents at the positions 2,3 a 4 on one hand, and β-D-ribonucleoside residue linked at the position N9 on the other hand. Compounds with other combinations of substituents at the 6-aryl group, or (aryl)-substituted purines do not exhibit any cytostatic activity, or their cytostatic activity is substantially lower compared to compounds of the general formula (I).

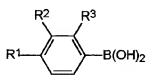
Compounds of general formula I can be prepared by general procedure (Suzuki reaction), consisting of a treatment of sugar-protected 6-chloropurine 9-β-D-ribonucleoside (II) with substituted phenylboronic acid in the presence of tetrakis(triphenylphosphine)palladium and a subsequent removal of the protecting groups at the sugar residue of the resulting intermediate.

Thus, easily accessible 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine (II),



(II)

is treated with phenylboronic acid of the general formula III

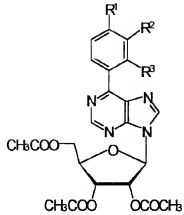


(III)

wherein R^1, R^2 and R^3 are identical with their meaning in formula I

in excess of 1.1 to 2 molar equivalents referred to compound II, in the presence of catalytic amount (5-10 mol. % referred to compound II) of tetrakis-(triphenylphosphine)palladium, preferably in toluene, in an inert atmosphere, at

temperature range of 80-100 °C, the thus-obtained peracetylated intermediate of the general formula IV



(IV)

wherein the meaning of R^1-R^3 is identical with those in formula I

is isolated, preferably by silica gel chromatography, and converted to compound of the general formula I preferably by treatment with sodium methoxide in methanol. Compounds of the general formula I usually crystallize directly from the reaction mixture in sufficient purity or can be further purified by different techniques, preferably by crystallisation of the deionized reaction mixtures from ethanol.

The required protected nucleoside of the formula II can be prepared by acetylation of easily commercially available nucleoside inosine and by reaction of thus-formed triacetate in chloroform solution with thionyl chloride. The boronic acids of the general formula III as well as the organometallic catalyst are commercially available. The yields of the intermediates of the general formula III are 65-90%, and their deacetylation is quantitative. Thus, the compound of the general formula I is obtained in the yield of 80-90%. Boric acid formed as the side-product of this reaction is ecologically acceptable.

Cytostatic effect of compounds is routinely examined *in vitro* in tissue cultures of transformed cells passaged in cell lines. Standardized "collection cultures" are used for the purpose. The examples of such routinely used cell lines are the following: transformed mouse leukemia cell line L-1210, immortalized line of the cells of chemically induced cervix carcinoma (HeLa) and immortalized human lymfoblastoid cell line (CCRF-CEM). After the inoculation of the culture medium by

the cells of the respective cell line the culture is left to grow before adding the examined compound to a certain cell density. The compound is added in an exponential growth phase and the number of cells per mL is counted at time intervals. This value is compared with the control, i.e. with the number of cells per mL growing in the absence of the test compound. The cytostatic activity IC_{50} of a test compound represents concentration of compound which suppresses by 50% the cell culture growth compared to the control cell culture. The IC_{50} value for a compound differs in different cell lines according to their sensitivity to a particular compound.

The invention will be further described by the following nonlimiting examples of the preparation of the claimed compounds of the general formula I and by the non-limiting examples of the determination of their biological activity.

Examples

Example 1

6-(4-Fluorophenyl)-9-(β-D-ribofuranosyl)purine.

413 mg (1.0 mmol) 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine, 210 mg (1.5 mmol) 4-fluorophenylboronic acid, 200 mg (1.5 mmol) potassium carbonate and 59 mg (0.05 mmol) $Pd(Ph_3P)_4$ are placed in a 50-mL flask equipped with reflux condenser with drying tube and a magnetic stirrer. 10 mL toluene is added, the apparatus is washed by argon and the mixture is heated under stirring 100 ^{0}C 8 h. The mixture is cooled and the solvent stripped in vacuo. The residue is chromatographed on a silica gel column (50 g) by ethyl acetate - petroleum ether mixture (1:2 - 9:1). The elution is monitored by TLC. The appropriate fractions are collected, evaporated and dried in vacuo to afford 410 mg (87%) 6-(4-fluorophenyl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine as amorphous foam. The yields and characteristics of additional compounds of the general formula IV obtained by this procedure are listed in Table 1.

Solution of the former compound (360 mg, 0.76 mmol) in methanol (20 mL) is treated by sodium methoxide in methanol (1M, 200 μL, 0.2 mmol) and the mixture is stirred in a closed flask overnight at ambient temperature. The separated crystalline material is collected by suction, washed with methanol and dried. The filtrate is neutralized by an addition of Dowex 50 X 8 (in H^+-form), filtered and the

resin washed subsequently with saturated ammonia solution in methanol (5 mL) and methanol (20 mL). The combined filtrates are evaporated *in vacuo* and the residue is combined with the first crystalline crop is recrystallized from ethanol. Yield, 220 mg (83 %) 6-(4-fluorophenyl)-9-(β-D-ribofuranosyl)purine, m.p.207-210 °C. FAB MS, *m/z* 347. Yields and characteristics of additional compounds of the general formula I obtained by this procedure are listed in Table 2.

Example 2

Determination of cytostatic effect on CCRF-CEM cells.

10^5 CCRF-CEM cells (human T lymphoblastoid cells, ATCC CCL119) are seeded in 0,9 mL RPMI 1640 medium with 10% calf phetal serum, L-glutamine (0,3 $g.l^{-1}$), 100 U/mL penicillin and 0,1 mg/mL streptomycin, placed in 24-well microtiter plates. The cells are cultivated in CO_2 incubator (37 °C), after 24 h the cell number is estimated in cell-counter 150+ and the test compound (dissolved in 0,1 mL physiological solution buffered with phosphate buffer pH 7,4) is added to final concentration 10 $\mu mol.L^{-1}$. The control wells are treated solely with physiological solution buffered with phosphate buffer pH 7,4). The cells are further incubated for 48 h and the final cell number is estimated. The inhibitory activity is expressed as % related to the final number of the cells in the control wells. The IC_{50} is determined from at least five different compound concentrations. The data shown in Table 3 are an average of four independent determinations for each compound. The cytostatic activity on L1210 cells is performed similarly.

Example 3

Determination of cytostatic effect on HeLa cells.

10^5 HeLa S3 cells (human epithelial cervical carcinoma, ATCC CCL 2.2) are seeded in 1 mL RPMI 1640 HEPES medium with 5% phetal calf serum, 100 U/mL penicillin a 0,1 mg/mL streptomycin placed in 24-well microtiter plates. The cells are incubated in CO_2 incubator (37 °C) 24 h and the cultivation medium is replaced by the same volume of medium containing the test compound (final concentration, 10 $\mu mol.L^{-1}$). The control wells are treated with fresh medium only. The cells are further incubated for 48 h and the final cell number is estimated by methylene blue dyeing method (30 min treatment, unadsorbed dye is washed with water and the adsorbed dye is extracted with 1% Sarkosyl (5 h at 37 °C). The UV-absorbance of the extract is determined spectrophotometrically at 600 nm. This method consists in

quantitative determination of proteins from cells adhered to the well surface. The appropriate cell counts are deduced from calibration curves. Inhibitory activity of the test compound (IC_{50}) is estimated essentially as described in Example 2.

Table 1. Preparation and properties of compounds of the formula IV

R^1	R^2	R^3	Yield, %	*m/z* [a]
H	H	H	79	455
F	H	H	87	473
F	H	F	65	491
F	F	H	81	491
H	H	CH_3	89	469
H	CH_3O	H	74	485
CH_3	H	H	79	469
CH_3O	H	H	84	485
Cl	H	H	65	489
C_2H_5O	H	H	80	499

a) Mass spectrum - FAB

Table 2. Preparation and properties of compounds of the formula I

Number	R^1	R^2	R^3	Yield, %	M.p. °C	*m/z* [a]
1	H	H	H	66	228-230	329
2	F	H	H	83	207-210	347
3	F	H	F	91	89-92	365
4	F	F	H	93	189-191	365
5	H	H	CH_3	91	79-81	343
6	H	CH_3O	H	71	141-143	359
7	CH_3	H	H	76	226-229	343
8	CH_3O	H	H	91	173-175	359
9	Cl	H	H	82	206-208	363
10	C_2H_5O	H	H	89	173-176	373

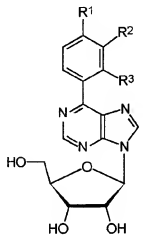
[a] Mass spectrum - FAB

Table 3. Cytostatic effect of compounds of the formula I

Compound				IC_{50} (μmol/L)		
Number	R^1	R^2	R^3	L-1210	HeLa	CCRF-CEM
1	H	H	H	9.0	2.7	0.7
2	F	H	H	4.5	2.5	0.75
3	F	H	F	NA	NA	NA
4	F	F	H	20.0	2.5	1.4
5	H	H	CH_3	NA	NA	NA
6	H	CH_3O	H	NA	NA	4.8
7	CH_3	H	H	NA	NA	1.5
8	CH_3O	H	H	1.5	4.3	0.25
9	Cl	H	H	NA	5.0	0.9
10	C_2H_5O	H	H	2.5	5.0	0.6

Claims

1. Novel 6-phenylpurine 9-β-D-ribonucleosides with antineoplastic activity of the general formula (I),



(I)

wherein R^1 is H, methyl, fluoro, chloro or alkoxy (C1-C2) group, R^2 is H, methyl or fluoro group, and R^3 is H, fluoro or methoxy group.

2. Pharmaceutical compositions containing compounds of the general formula (I) according to Claim 1 as active components.

3. The use of compounds of the general formula (I) according to Claim 1, or their combinations, for preparing pharmaceutical compositions for the treatment of cancer and leukemia.